

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1(Currently amended). A method for screening and identifying molecules that transactivate a neurotrophin receptor and mediate neuronal cell survival in the absence of neurotrophins, comprising ~~one or a combination of assays A, B or C~~ assay A or assay A in combination with either or both of assay B and assay C, wherein:

assay A comprises:

treating neuronal cells with a candidate small molecule activator;

reacting a neurotrophic receptor, obtained from a cell lysate of the treated neuronal cells, with an anti-phosphotyrosine antibody specific for a phosphorylated form of the neurotrophin receptor; and

detecting specific binding of the anti-phosphotyrosine antibody to a phosphorylated form of the neurotrophin receptor to identify a small molecule activator of the neurotrophin receptor;

assay B comprises:

treating neuronal cells with a candidate small molecule activator;

reacting either phosphatidylinositol 3'-kinase (PI3-K), obtained from a cell lysate of the treated neuronal cells, with an anti-phospho-PI3-K antibody specific for the phosphorylated form of PI3-K or Akt, obtained from a cell lysate of the treated neuronal cells, with an anti-phospho-Akt antibody specific for the phosphorylated form of Akt; and

detecting specific binding of the anti-phospho-PI3-K antibody to the phosphorylated form of PI3-K or of the anti-phospho-Akt antibody to the phosphorylated form of Akt to identify a small molecule activator of a neurotrophin receptor and its downstream Akt target; and

assay C comprises:

culturing neuronal cells in the presence of neurotrophins;

treating and culturing the neuronal cells with a candidate small molecule activator in the absence of neurotrophins; and

determining the level of cell survival to identify a small molecule activator of the neurotrophin receptor.

2(Original). The method of claim 1, wherein the neurotrophin receptor is a Trk receptor.

3(Original). The method of claim 2, wherein the Trk receptor is TrkA receptor.

4(Original). The method of claim 3, wherein the neuronal cells are PC12 neuronal cells.

5(Original). The method of claim 3, wherein, in assay A, the anti-phosphotyrosine antibody is specific for a phosphorylated tyrosine residue 684 of TrkA.

6(Original). The method of claim 1, wherein the candidate small molecule activator is a ligand of a G protein coupled receptor (GPCR).

7(Original). The method of claim 1, wherein the neurotrophin receptor is a Ret receptor.

8(Original). The method of claim 7, wherein the neuronal cells are N2a neuroblastoma cells.

9(Original). The method of claim 1, wherein, in the reacting and detecting steps of assay B, Akt is reacted with anti-phospho-Akt antibody and specific binding of anti-phospho-Akt antibody to the phosphorylated form of Akt is detected.

10(Original). The method of claim 9, wherein assay B further comprises:

reacting Akt, obtained from a cell lysate of the treated neuronal cells, with an anti-Akt antibody; and

detecting specific binding of the anti-Akt antibody to Akt to provide an assessment of the relative level of phosphophosylated Akt and the extent of activation.

11(Original). The method of claim 1, wherein in the reacting and detecting steps of assay B, PI3-K is reacted with anti-phospho-PI3-K antibody and specific binding of anti-phospho-PI3-K antibody to the phosphorylated form of PI3-K is detected.

12(Original). The method of claim 11, wherein assay B further comprises:

reacting PI3-K, obtained from a cell lysate of the treated neuronal cells, with an anti-PI3-K antibody; and

detecting specific binding of the anti-PI3-K antibody to PI3-K to provide an assessment of the relative level of phosphophosphorylated PI3-K and the extent of activation.